

Segmentation

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What are segments?

Repeated units of anatomical but not necessarily biochemical identity.

Segments develop from unsegmented tissue and acquire strict borders.

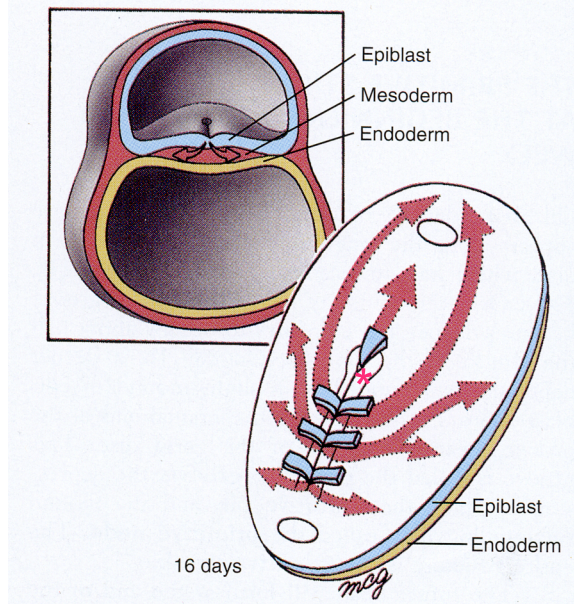
A “no mixing rule” contributes to segment identity.

In vertebrates these segments are derived from the paraxial mesoderm and are called somites.

Topics

1. Segmentation of trunk mesoderm: the derivatives of paraxial mesoderm = the somites.
 - A. Cells of origin of somites.
 - B. Follow anatomical changes and molecular underlying.
 - C. Imposition of segmentation on trunk PNS.
 - D. Concept of homeotic transformations and hox (homeobox) genes.
2. Segmentation CNS – example the rhombomeres of the hindbrain.

Origin of somites

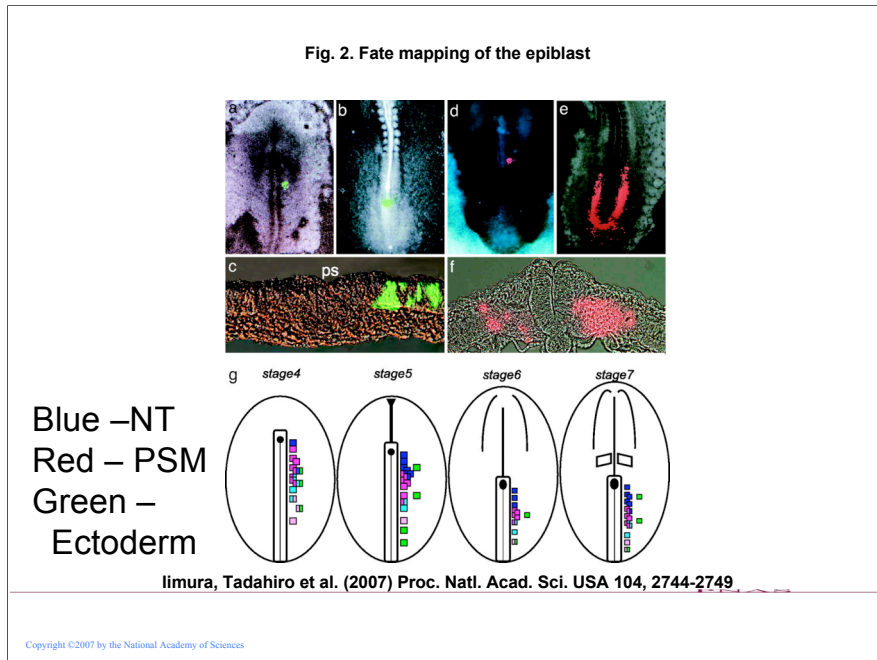


Earlier in the course we studied the gastrulation movements of epiblast cells into and through the primitive node and streak. The cells that give rise to the **axial** mesoderm (notochord) enter via the primitive node. Those that enter just caudalward (red asterick) give rise to the **paraxial** mesoderm which will, for the most part, become segmented.



Arrows point to boundary of pre-somitic mesoderm and forming somites.

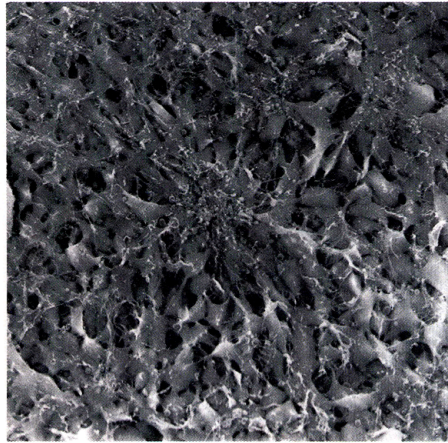
Fig. 2. Fate mapping of the epiblast



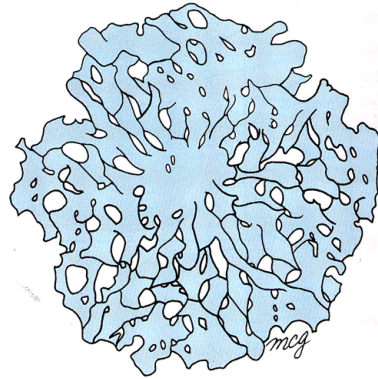
The skeletal muscles and axial skeleton of vertebrates derive from the embryonic paraxial mesoderm. Competitive hypotheses. Does paraxial mesoderm arise from stem cells in primitive streak? In **a-c** epiblast cells from a donor are labeled with EGFP and then transplanted. Epiblast fate mapping using Dil labeling *in ovo*. Epiblast cells adjacent to the anterior primitive streak level of the embryo shown in **e**. This experiment shows that epiblast cells *in ovo* act like graft.

(g) Summary of the results of the epiblast fate mapping. Each square indicates the fate of an individual cell: blue, neural tube; red, paraxial mesoderm; green, surface ectoderm. Using this approach they found that the paraxial mesoderm territory in the epiblast is largely conserved.

Scanning EM and diagram of a somitomere: organized whorl of cells that **prefigure** the somites.



A

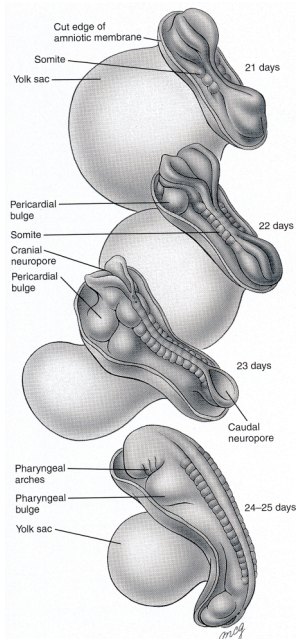


B

The segmentation of the paraxial mesoderm is pre-figured by the coming together of whorls of cells as illustrated here. All of the somitomeres except for the 7 most cranial become somites.

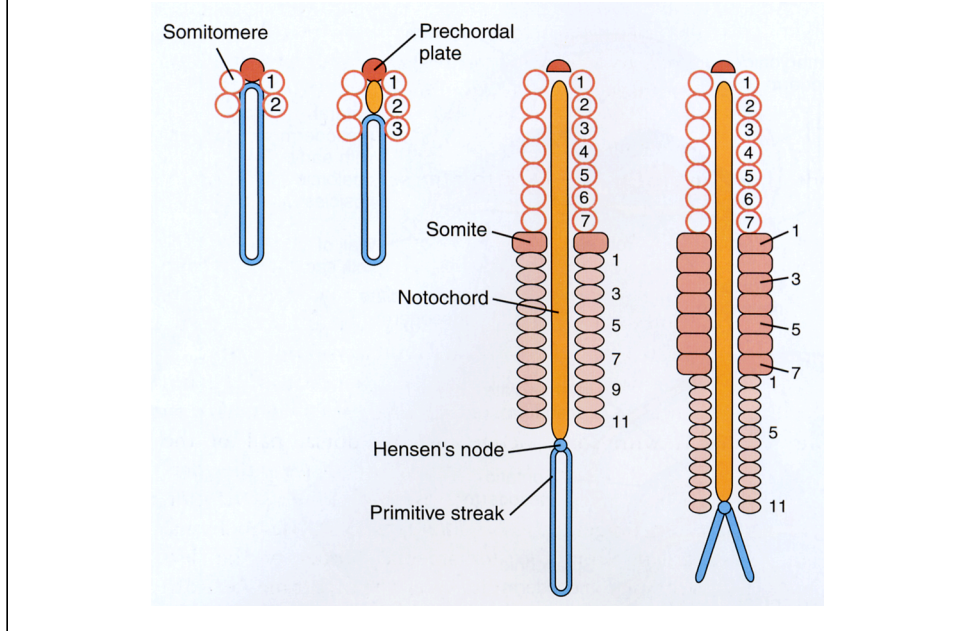
Sequential addition of somites from day 21- day 25 post-conception

Humans are estimated to have 42-44 pairs



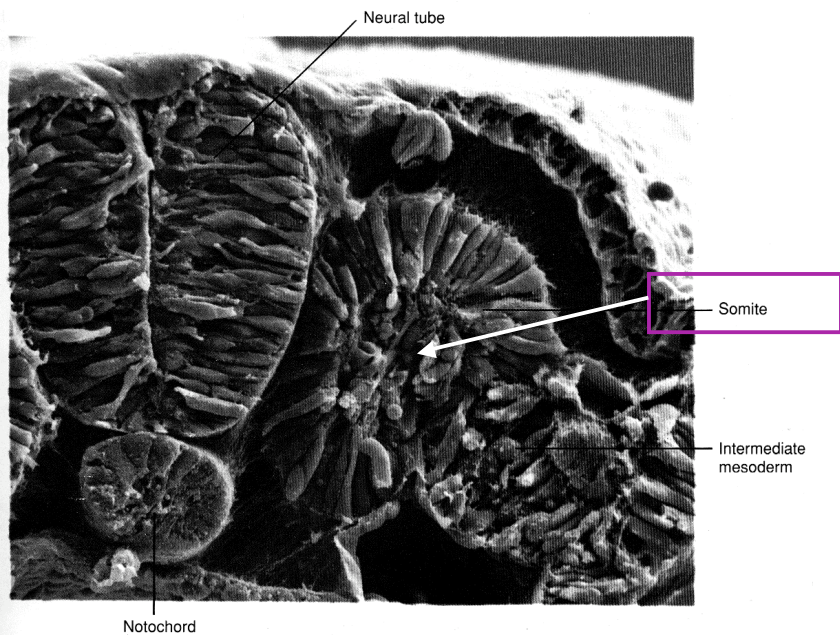
Cranial to caudal appearance and disappearance. Not all somites are present simultaneously.

Sequential appearance of somitomeres and somites



Sequential addition of somitomeres and somite transformation as node "retreats".

Scanning EM of the epithelial somite.



Further differentiation of the somites requires specific changes in gene expression. For example, the initial formation of the epithelial structure of the somite is marked by an increase in cellular adhesion molecules, predominantly E-cadherin, and the addition of the extracellular matrix protein, fibronectin, to the components of the basal lamina. To continue differentiation, the cells must de-epithelialize by down-regulating E-cadherin expression, resume a mesenchymal phenotype, and migrate.

Interdependence of tissues in somite formation.

1. Pre-somitic mesoderm will form somites in a **cell autonomous fashion**. **However they do not progress normally without their tissue neighbors.**
2. Removal neural tube - the vertebrae that develop lack segmentation of the dorsal structures (i.e. the neural arch).
3. Removal of notochord - the ventral half of the vertebrae, the vertebral bodies, lose segmentation and bilaterality so that they fuse ventral to the neural tube.
4. Removal of notochord and floor plate (specialized cells on the midline of the neural tube) and transplantation dorsal to the somite, completely inhibits muscle formation; the myotome differentiates into cartilage.

1. Most of the somitomers will develop into epithelial rosettes, the somites, which can be seen clearly through the covering ectoderm
2. As the segmental boundaries are established, cells from one somite will not cross into another.
3. Somites decondense (undergo epithelial to mesenchymal transformation) soon after they are formed so that not all somites are present at one time.

Somite undergoes epithelial to mesenchymal transformation (down regulate adhesion factors).

Somite has territories.

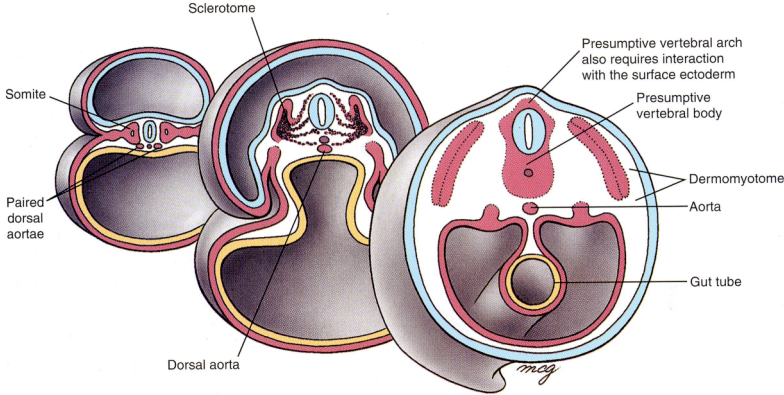
Medial = sclerotome = vertebrae

Lateral = dermamyotome

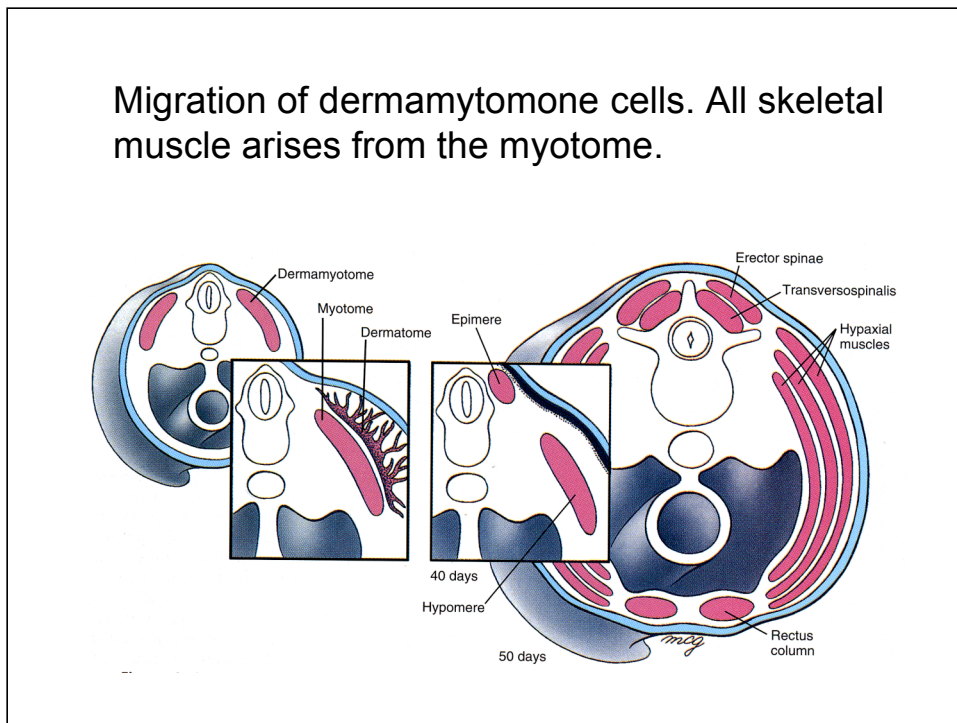
- dermatome = dermis derived from most lateral aspect.

- myotome = epimere and hypomere = segmented muscles and muscles of the limb.

Fate of the sclerotome.



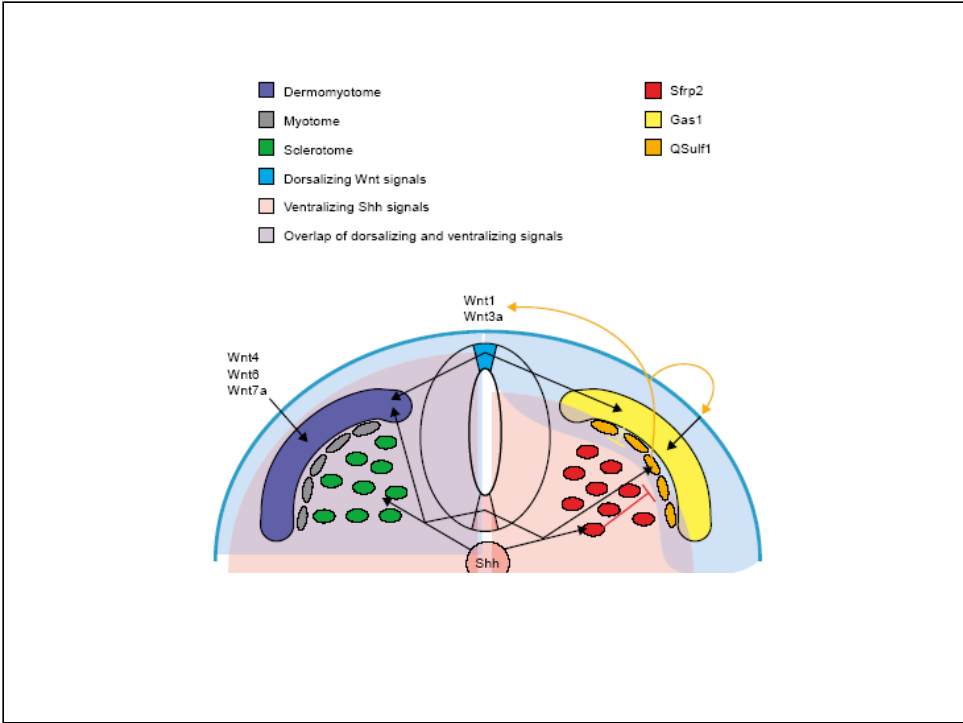
Migration of dermamyotome cells. All skeletal muscle arises from the myotome.



Dorsolateral dermatome and medial ventral myotome. Dermatome cells migrate to ectoderm at segmental level, collaborate with LPM to form dermis. Myotome to epimere and hypomere. Epi, deep muscles of back including the erector spinae and transversospinalis groups. Thoracic hypomere 3 layers of anterior lateral muscle of lateral and ventral body wall in thorax and abdomen. Intercostals in the thorax and external oblique, internal oblique and transversus abdominis and rectus abdominis muscles that flank midline. Rectus column is usually confined to abdomen but may sneak up to thorax as sternalis muscle. Page 85 in text. Tendons and internal connective tissue of muscle from somatopleure.

TABLE 6-1 *Subdivisions of the Epithelial Somite*

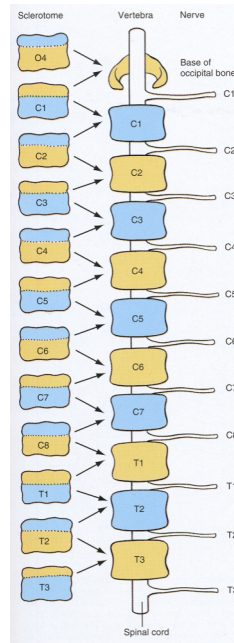
Dorsal		
DERMATOME		DERMATOME
Dermis		Dermis
Myotome		Myotome
Intrinsic back muscles (epaxial)		Limb muscles
		Muscles of ventrolateral body wall
MEDIAL	SOMITOCOEL CELLS	LATERAL
	Intervertebral joint surfaces	
SCLEROTOME		SCLEROTOME
Vertebral body		Vertebral arch
Intervertebral disk		Pedicle of vertebra
Proximal part of rib		Distal part of rib
Connective tissue		Connective tissue around dorsal root ganglion
	Ventral	



Positional information:
Transplantation of somites

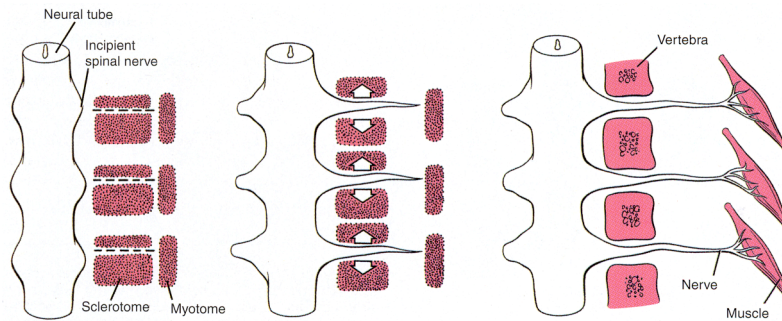
If thoracic somites are moved to the cervical region before they de-epithelialize, the transplant will still give rise to ribs.

Each vertebra is formed from the caudal portion of one somite and the cranial portion of the next. Note origin of spinal nerve C1.

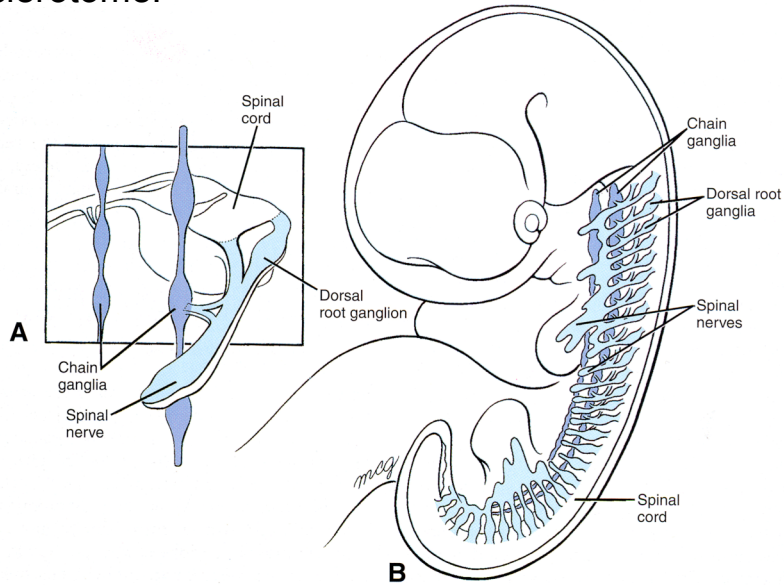


1 nerve emerges from anterior 1/2 of C1 but this half becomes part of occipital bone.

Each spinal nerve traverses the cranial aspect of the sclerotome as it grows to innervate the myotome.
The segmental nature of the PNS is due to segmentation of somites.



Neural crest migrate through cranial $\frac{1}{2}$ of sclerotome.



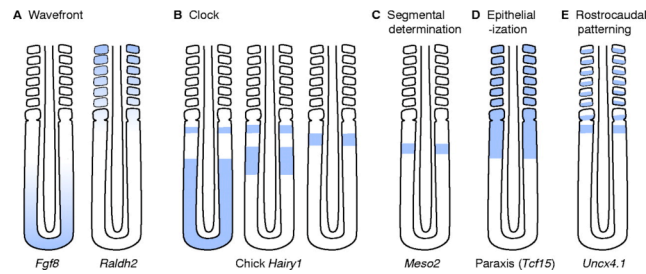


Arrows point to boundary of pre-somitic mesoderm and forming somites.

Molecular aspects of somitogenesis.

Somitogenesis, the sequential formation of a periodic pattern along the anterior-posterior axis of vertebrate embryos, is one of the most obvious examples of the segmental patterning processes that take place during embryogenesis and also one of the major unresolved events in developmental biology.

Categories of gene expression patterns that are associated with paraxial mesoderm segmentation and maturation in the chick embryo



Dubrulle, J. et al. *Development* 2004;131:5783-5793

The figures A-E above indicate the sequence of events leading to full differentiation of the chick embryo. (A-E) In situ hybridization gene expression patterns in the presomitic mesoderm (A) show a gradient of gene expression (left to right) associated with the retinoic acid (retaldehyde dehydrogenase 2) gradient (right). (B) Expression of a cyclic gene via notch signaling (Chick Hairy1) is associated with segmental compartmentalization, such as these belonging to the *Mesp/Meso2/Thylacine1* bHLH transcription factors. (E) Genes highlighting the rostrocaudal compartmentalization of the formed somites (Uncx4.1).

Determination of psm

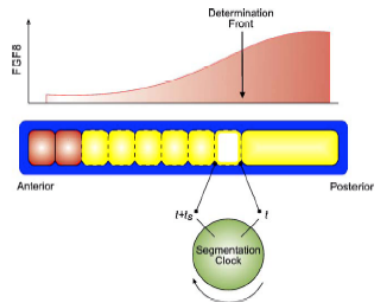
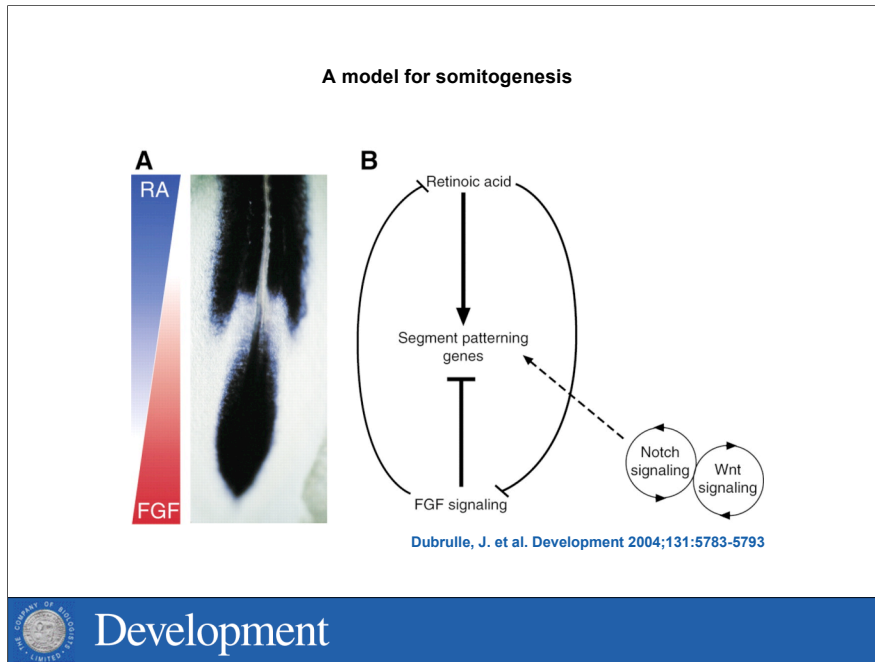


Fig. 2. Diagrammatic representation of the vertebrate body plan during somite formation within Pouquié's Clock and Wavefront model. In the top part of the diagram, the FGF8 wavefront is illustrated together with the position of the determination front. The middle section of the diagram shows the AP axis of the embryo with the somites (red blocks), determined region and its pre-pattern (yellow blocks) and the undetermined PSM (yellow band) clearly marked. The bottom part of the diagram shows the segmentation clock with the time t at which a cell reaches the determination front and the time t_0 later at which it becomes competent to signal. The hollow yellow block marks the position of the next somite to be specified; the posterior boundary is fixed by the position of the determination front at the time at which pioneer cells at the anterior boundary produce a signal.

Dynamic expression of FGF8 in the presomitic mesoderm constitutes the wavefront of determination which sweeps along the body axis interacting as it moves with the segmentation clock to gate cells into somites.



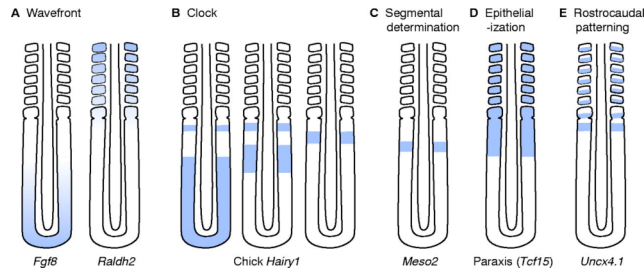
A model for somitogenesis. (A) Double in situ hybridization of a 2-day-old chicken embryo shows mutually inhibitory, antagonistic gradients of retinoic acid (RA) and fibroblast growth factor (FGF). (B) Schematic diagram of the model showing that RA activates segment patterning genes, which in turn inhibit FGF signaling. FGF signaling inhibits RA. Notch and Wnt signaling pathways are mutually inhibitory and also inhibit segment patterning genes.

What governs addition of somites from somitomeres?

There is a molecular oscillator known as the segmentation clock which drives the periodic transcription of cyclic genes in the presomitic mesoderm.

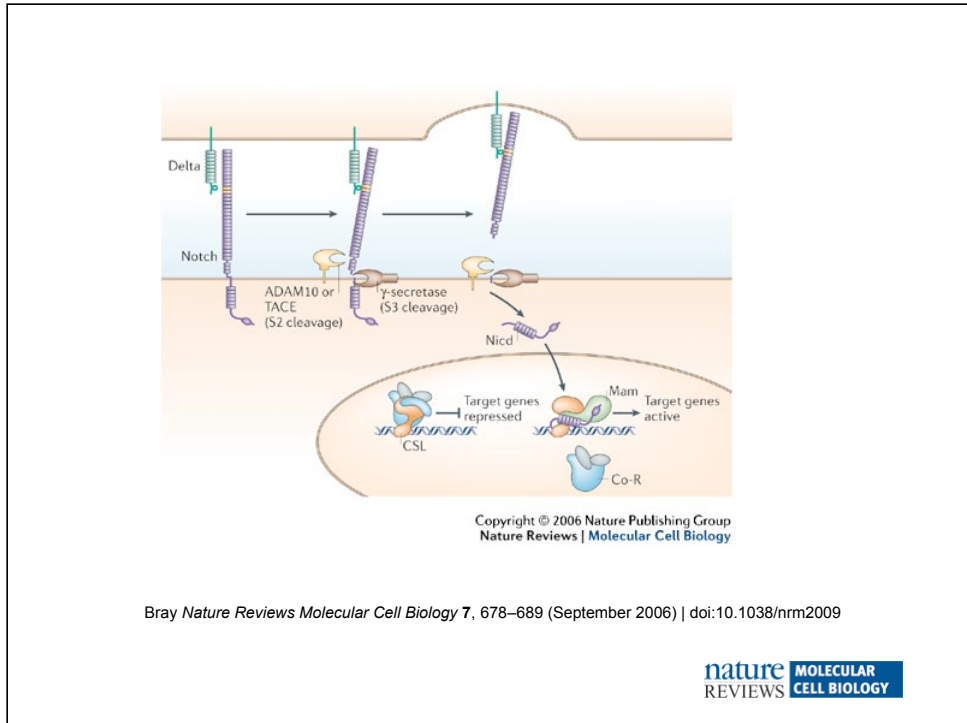
The genes involved to date are downstream targets of Notch (*aka hairy*) or are regulators of Notch signaling (*aka Lunatic fringe*).

Categories of gene expression patterns that are associated with paraxial mesoderm segmentation and maturation in the chick embryo



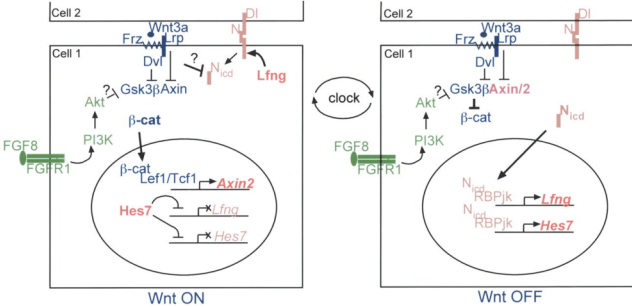
Dubrulle, J. et al. *Development* 2004;131:5783-5793

The figures A-E above indicate the sequence of events leading to full differentiation of the chick embryo. (A-E) In situ hybridization gene expression patterns in the presomitic mesoderm (A) show a gradient of *Fgf8* and *Raldh2* at the posterior end. (B) Expression of a cyclic gene via notch signaling, such as *Chick Hairy1*. (C) Segmental determination, such as *Meso2*. (D) Epithelialization, such as *Paraxis (Tcf15)*. (E) Genes highlighting the rostrocaudal compartmentalization of the formed somites, such as *Uncx4.1*.



There is compelling genetic evidence showing that Notch–Delta signaling is indispensable for somitogenesis. Notch receptor and its target genes, *Hairy/E(spl)* homologues, are known to be crucial for the ticking of the segmentation clock.

Figure 3. Model of the molecular clockwork

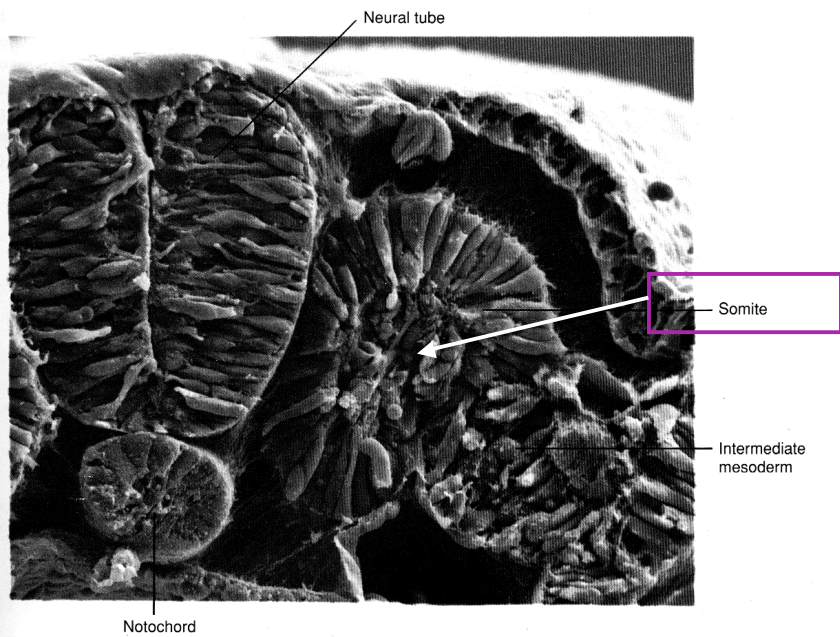


Alexander Aulehla et al. *Genes Dev.* 2004; 18: 2060-2067

Generation of form and
diversity: homeotic
transformations

Or: Not all somites are created
equal

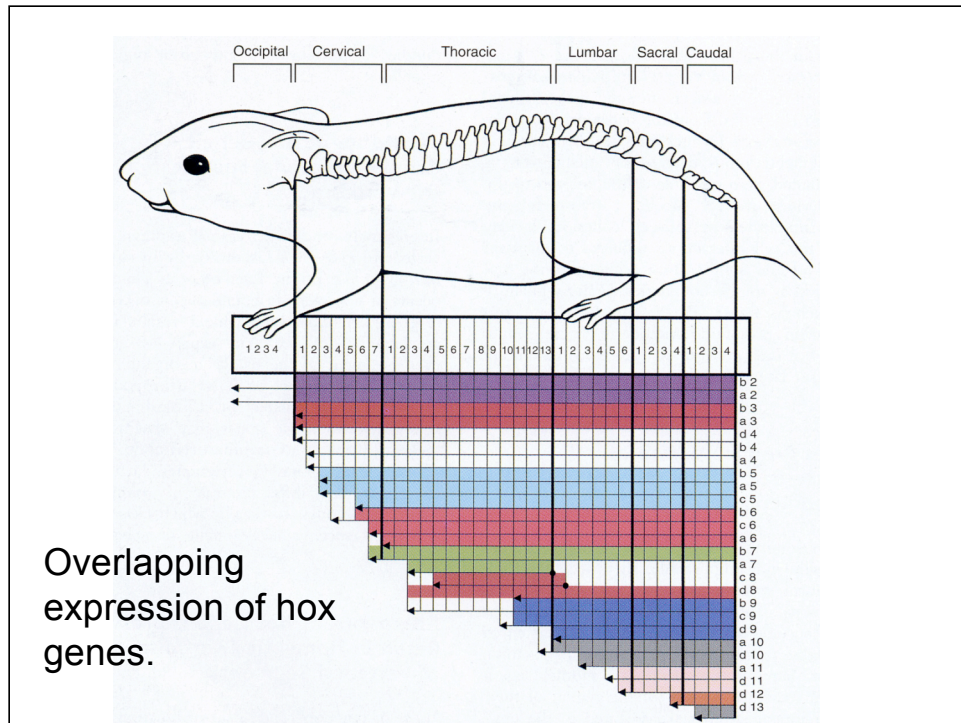
Scanning EM of the epithelial somite.



Generation of diversity.

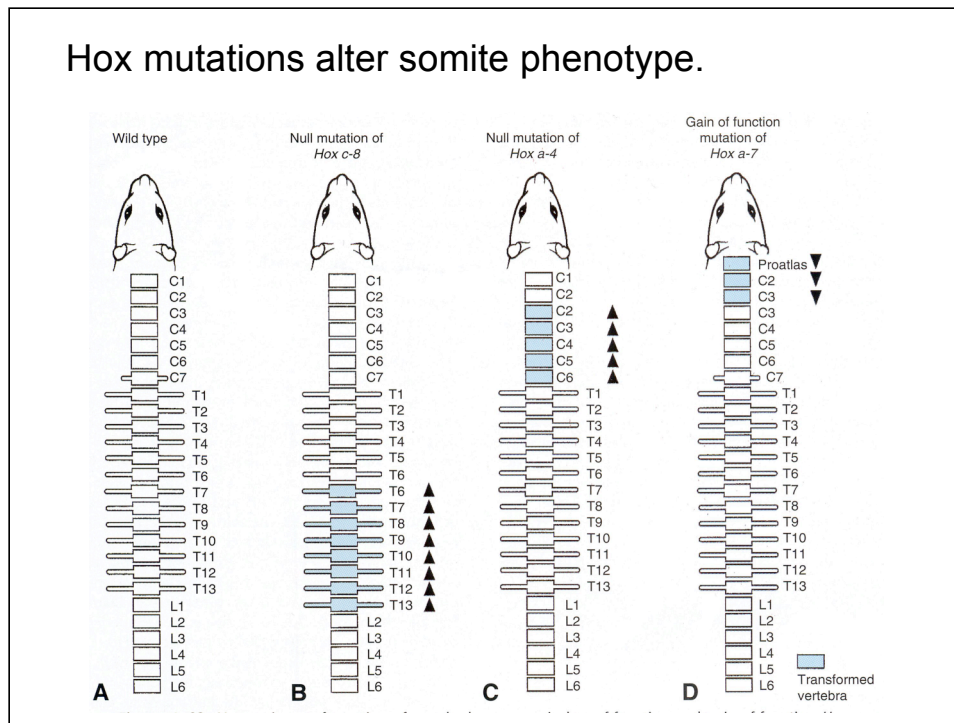
- 1. Establish a segmental pattern followed by homeotic transformations of each segment.**
- 2. Homeotic transformation is the differentiation of initially identical repeating segments into unique structures; the nature of the homeotic transformation is dependent on the expression pattern of homeotic genes = HOX genes.**
- 3. This strategy is conserved throughout the animal kingdom and vertebrates are recognized as a separate animal phylum because of their most conspicuous segmentation, the vertebral column.**

One of the mechanisms by which animals generate form and diversity is first to establish a segmental pattern followed by homeotic transformations of each segment. Homeotic transformation is the differentiation of initially identical repeating segments into unique structures; the nature of the homeotic transformation is dependent on the expression pattern of homeotic genes. This strategy is conserved throughout the animal kingdom and vertebrates are recognized as a separate animal phylum because of their most conspicuous segmentation, the vertebral column.



A **homeodomain** is a structural motif found in transcription factors involved in (amongst other things) the expression of developmentally regulated genes. These transcription factors are encoded by **homeotic genes**. The **homeodomain** is a variation on a helix-turn-helix motif; 3 alpha-helical regions separated by turns in the protein backbone. One of these alpha helices acts to **recognize specific DNA sequences in the promotor / enhancer region** of the genes which they regulate (binding to the major groove of the DNA), while the other two serve to help position the "recognition helix".

Hox mutations alter somite phenotype.



Homeotic transformation of vertebral segments in loss-of-function and gain-of-function *Hox* genes in mice. Null mutations of *Hoxc-8* (compare **B** to **A**) and *Hoxa-4* (compare **C** to **A**) cranialize vertebrae within their normal expression domains. A gain-of-function mutation of *Hoxa-7* caudalizes vertebrae outside its normal expression domain (compare **D** to **A**).

Summary:

1. Somites establish body segmentation.
2. Somite has 3 separate compartments.
3. Somites are responsible for the segmentation of the vertebral column, PNS, segmental muscles.
4. Overlapping patterns of HOX gene expression result in somites with individual characteristics.
5. Positional information is present in somites prior to epithelial-mesenchymal transformation perhaps due to gradients of fgf8 and or RA.

The end.